EFFECTS OF TWO MUSTARD OILS ON PLASMODIOPHORA BRASSICAE AND THEIR RELATION TO RESISTANCE TO CLUBROOT[1]

By W. J. HOOKER, *formerly instructor*, and J. C. WALKER, *professor, Department of Plant Pathology*,[2] and KARL PAUL LINK, *professor, Department of Biochemistry, Wisconsin Agricultural Experiment Station.*

INTRODUCTION

The possible effects of mustard oils on the development of clubroot (*Plasmodiophora brassicae* Wor.) of crucifers were first suggested by Rochlin (Rokhlina) (*14*, 15).[3] She concluded that resistance was associated with the presence of glucosides yielding mustard oils upon hydrolysis. When cabbage (*Brassica oleracea* var. *capitata* L.) seedlings grown in heavily infested soil were watered with the aqueous extracts of seeds of black mustard (*B. nigra* (L.) Koch), only 20 percent became diseased, while in untreated controls 100 percent were infected. The toxic properties of mustard oils to various bacteria and fungi have been demonstrated (*3*, *6*, *7*, *11*, *13*, *19*).

When Pryor (*12*) grew turnip (*Brassica rapa* L.), black mustard, and cabbage in sand cultures without sulfur in the nutrient solution the plants were found to be very low in mustard-oil content, yet their relative resistance or susceptibility to clubroot was only slightly altered. He concluded that factors other than mustard oils were chiefly responsible for resistance. Stahmann, Link, and Walker (*17*) isolated beta-phenethyl isothiocyanate from the root tissues of resistant and susceptible strains of turnip and black mustard, from a susceptible strain of white mustard (*B. hirta* Moench (*B. alba* (L.) Boiss.)), and from susceptible cabbage, but the presence of allyl isothiocyanate could not be detected. The roots of horseradish, (*Armoracia rusticana* Gaertn., Mey., and Scherb.) contained both beta-phenethyl isothiocyanate and allyl isothiocyanate. These investigators were unable to correlate resistance with either mustard-oil content or myrosin activity in these hosts.

The investigation reported here consisted of a study of the effects of the two mustard oils, allyl isothiocyanate ($CH_2=CH—CH_2—N=C=S$) and beta-phenethyl isothiocyanate ($C_6H_5—CH_2—CH_2—N=C=S$), on the germination and infectivity of the clubroot organism.

MATERIALS AND METHODS

Since *Plasmodiophora brassicae* is an obligate parasite and since clubroot tissue is rapidly invaded by secondary organisms, it is difficult to obtain suspensions of resting spores entirely free from other

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[2] Also agent, Division of Fruit and Vegetable Crops and Diseases, Bureau Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[3] Italic numbers in parentheses refer to Literature Cited, p. 77.

organisms. In these investigations spore suspensions were purified as much as possible after the method described by Wellman (*20*). Firm clubs from diseased plants were washed, frozen at −4° C., and stored in the frozen state until used. Spores were separated from host debris by first macerating diseased tissue in a meat chopper or Waring blendor and then filtering through cheesecloth with the spores remaining in the filtrate. Small pieces of host tissue passing through the cheesecloth were separated from the spores by centrifuging for about 15 seconds. The supernatant liquid containing spores was then centrifuged in a Sharples supercentrifuge. The puttylike mass of spores was suspended in distilled water and the process repeated until the spores had been carried through at least three complete changes of relatively large volumes of water. Purified spore suspensions were either held overnight at 8° C., and diluted the following day for spore germination studies, or frozen and held until used. Throughout the series of investigations, unless otherwise specified, the resting-spore concentration was maintained at approximately 25,000 spores per cubic millimeter.

Mustard-oil solutions were prepared from stocks obtained in 1938 and stored continuously at 8° C. Allyl isothiocyanate, practical grade, was purchased from a camera company; beta-phenethyl isothiocyanate was synthesized after the method described by Stahmann, Link, and Walker (*17*). Because of the low solubility of the phenethyl oil in water, mustard-oil solutions were prepared by the addition of aliquots of a stock solution of oil in ethyl alcohol to distilled water. Since these oils react slowly with ethyl alcohol, stock solutions were prepared and stored at 8° C. generally 12 hours before the final dilution.[4] The final ethyl-alcohol concentration, never exceeding 1 percent by volume, was usually 0.7 percent. Equal volumes of mustard-oil solutions as well as spore suspensions prepared at twice the desired concentrations were thoroughly mixed, resulting in spore suspensions and oil solutions of a given concentration.

The effects of mustard oils on zoospore formation were observed in aqueous mustard-oil solutions of 50 ml. contained in 125 ml. Erlenmeyer flasks tightly stoppered with a tin-foil-covered cork. Flasks were stored in the dark at 23° C. Daily observations of zoospores were made by means of a Levy blood-counting chamber. The flasks were vigorously shaken before sampling and a drop of spore suspension was removed from the solution with a 5-mm. loop of nichrome wire. Zoospore counts were made by determining the number of zoospores in 15 fields each containing 1/250 mm^3. Five fields were examined from each sample and three samples were removed from each flask.

The effects of the two oils upon *P. brassicae* were determined in a different manner by the amount of infection and disease development on cabbage, which has been shown to be very susceptible to the organism (*18*). Healthy cabbage plants, 3 to 4 inches high, of the yellow-resistant variety Jersey Queen, were planted into clubroot-free, moist, muck soil which had been adjusted to pH 5.4. The soil was first packed into 4-inch clay pots and five holes per pot formed in the soil with a glass rod to facilitate planting. A 5-ml. aliquot of spore

[4] The writers are indebted to Mark A. Stahmann and F. G. Smith, of the Department of Biochemistry, University of Wisconsin, for synthesis of beta-phenethyl isothiocyanate and for supplying oil solutions in concentrations suitable for th preparation of spore suspensions.

suspension in mustard-oil solution was placed with the plant in each of the five transplanting holes. The soil was firmly packed around the plants and well watered with distilled water. Since approximately 18 hours of high soil moisture is necessary for infection (*20*), the soil was kept as moist as possible for 3 to 4 days until infection had been established. To preclude excessive damping-off, the soil moisture was then gradually reduced and maintained at as low a level as was still compatible with good growth. The plants were grown for about 2 months, then removed and examined.

EXPERIMENTAL RESULTS

EFFECTS OF MUSTARD OILS ON GERMINATION OF PLASMODIOPHORA BRASSICAE

The effects of allyl and of beta-phenethyl isothiocyanate on spore germination were determined by direct zoospore counts. The course of spore germination in this study was in general agreement with the findings of Wellman (*20*), who observed that the rate of germination in water cultures gradually increased to a peak on the fourth day of incubation, after which it gradually decreased. Very little germination was observed during the first 2 days of incubation. In this study the time at which germination in distilled water reached a peak varied from the third to the fifth day, and sometimes the peak extended over a period from the fourth to the seventh day. It was usually impossible to make accurate zoospore counts after the seventh day because of the development of bacteria, protozoa, and filamentous fungi in the culture solution. Since it was necessary to use a small amount of ethyl alcohol in the preparation of the oil suspensions, controls were prepared in 0.7 percent solution of the former, a concentration slightly greater than that to which the spores were generally exposed. Since no consistent effect of alcohol upon germination was observed in the trials conducted, it was concluded that the effect of mustard oils upon germination was independent of the alcohol used in the preparation of the oil.

In the high concentrations the addition of mustard oils to the aqueous suspensions of spores was observed to have a marked inhibitory effect upon germination. The toxic levels at which spore germination was inhibited by each oil are shown in table 1. Original concentrations of the allyl oil inhibiting spore germination of *P. brassicae* ranged from 10 to 80 p. p. m., whereas original concentrations of beta-phenethyl isothiocyanate inhibiting visible germination ranged from 5 to 80 p. p. m. Whenever direct comparisons of the toxic levels of the oils could be made, no consistent difference was evident. In experiment 2, the phenethyl oil was more toxic than the allyl oil and in experiment 8 the reverse obtained. In all other trials the oils were approximately equal in toxicity when compared on a weight basis. However, since the beta-phenethyl oil is 1.7 times the molecular weight of the allyl oil, the former was almost twice as toxic when compared on a molecular basis.

In addition to the inhibitory effect exercised by the oils, marked stimulation of zoospore production was observed at oil concentrations below the inhibitory levels. The results of a typical experiment (experiment 2, table 1) in which direct comparisons could be made between the two oils on zoospore formation are shown in

TABLE 1.—*Effects of 2 mustard oils upon germination of the resting spores of Plasmodiophora brassicae, as determined by direct zoospore counts*

Experiment No.	Initial concentration completely inhibiting spore germination		Initial concentration stimulating spore germination	
	Isothiocyanate		Isothiocyanate	
	Allyl	Phenethyl	Allyl	Phenethyl
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
1	20.0		10.0	
2	10.0	5.0	2.5	1.25
3	10.0		1.25–5.0	
4	80.0	80.0	.6–10.0	1.25–10.0
5	80.0	(1)	40.0	(2)
6	80.0	80.0	40.0	(2)
7	20.0	20.0	10.0	(-)
8	40.0	80.0	20.0	40.0

[1] Toxic concentration not clearly defined.
[2] Germination not sufficient to indicate most favorable concentration.

figures 1 and 2. The allyl oil (fig. 1) was decidedly stimulatory to germination at initial concentrations of 1.25 and 2.5 p. p. m. The higher concentration more nearly approached the optimum in this experiment although good germination took place at both levels. On the other hand, it is evident that the phenethyl oil was toxic at 2.5 p. p. m., while at 1.25 p. p. m. it was more stimulatory than was the allyl form at 2.5 p. p. m.

The extent of germination and the level at which stimulation was greatest varied considerably from one experiment to another. This may have been due in part to variability in the spore suspensions

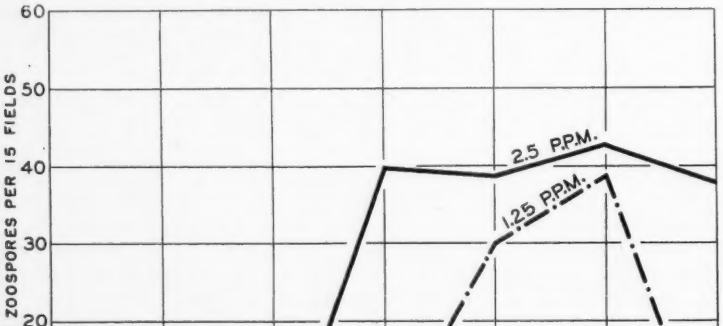

FIGURE 1.—Effect of allyl isothiocyanate at different concentrations on the germination of resting spores of *Plasmodiophora brassicae*. No germination was observed in this experiment at concentrations of 10 p. p. m. and higher.

which, although made up by uniform procedures, were from diseased plants collected at different times and in different locations. The beta-phenethyl oil stimulated germination at concentrations below the toxic level in three out of six trials, while the allyl was effective in each trial. However, when the former caused stimulation, the effect was more pronounced than with the allyl oil.

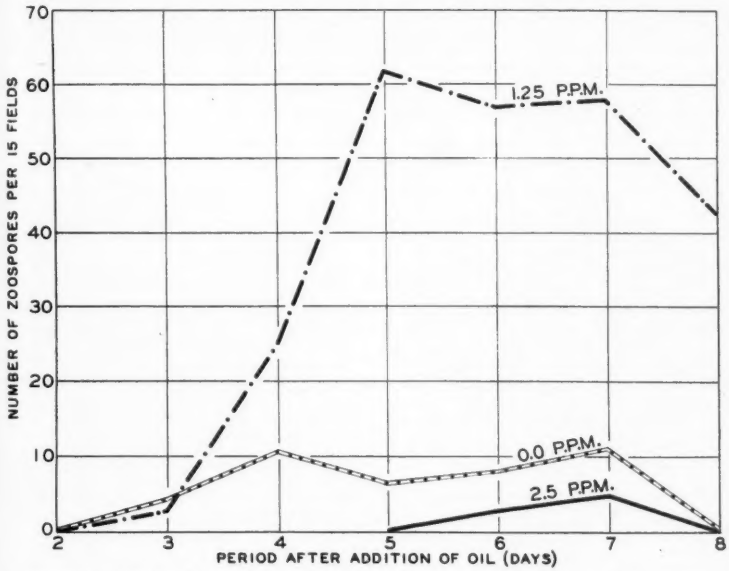

FIGURE 2.—Effect of beta-phenethyl isothiocyanate at different concentrations on the germination of resting spores of *Plasmoidophora brassicae*. No germination was observed in this experiment at concentrations of 5 p. p. m. and higher.

EFFECTS OF ALLYL ISOTHIOCYANATE ON COLLETOTRICHUM CIRCINANS AT SUBLETHAL LEVELS

Since the mustard oils stimulated spore germination of *Plasmodiophora brassicae*, a limited number of tests were conducted with a readily culturable fungus, *Colletotrichum circinans* (Berk.) Vogl., in order to determine whether or not the reaction to the oils was peculiar to *P. brassicae*. This organism was selected because the spores germinate readily in distilled water and because its reaction to mustard oils had been determined previously (*7*, *13*, *19*). Since the stimulatory effect on *P. brassicae* occurred more consistently with allyl isothiocyanate than with the beta-phenethyl oil, tests on germination of spores *C. circinans* were carried out with the first-mentioned oil.

Spore suspensions were prepared after the method of McCallan and Wilcoxon (*9*). Approximately 10 ml. of water was added to spores which had been gently scraped from the surface of an agar slant. The suspension was at once poured off into a sterile tube, filtered through a small amount of absorbent cotton, and centrifuged for 10 minutes after which the supernatant liquid was poured

off. The spores were taken up in distilled water and stored at 80° C. until used. Petri dishes adapted as moist chambers were used. Sterile glass slides were placed on U-shaped glass rods placed in the bottom of the dish. Approximately 2.5 ml. of oil and water of the desired concentration was placed in the bottom of the Petri dish. This oil reserve served to maintain a supply of oil vapor in the moist chamber during the period of germination. Oil solutions and spore suspensions at twice the desired concentration were thoroughly mixed and a 5-mm. loop of the suspension was placed on the glass slide. The Petri dishes were placed inside large moist chambers and stored at 23° C. until the spores had germinated.

The limitations of moist chambers of this type for the study of a volatile chemical are apparent. However, it was believed that a suitable range of concentrations was maintained over the relatively short period required for germination even though the initial concentration may have dropped as a result of the evaporation of oil. The ethyl alcohol concentration throughout the trials was maintained at 1.0 percent by volume.

Spore germination in 1.0 percent ethyl alcohol was generally very similar to that in distilled water, indicating that the solvent had little effect in the final reaction. Observations on spore germination made at the end of 5 hours showed no stimulatory effect of allyl isothiocyanate at subinhibitory concentrations upon spore germination. Previous trials, the results of which are not presented, had shown no stimulatory effect upon spore germination at subinhibitory levels after 12 hours' incubation. Allyl isothiocyanate in these incompletely closed systems prevented germination in all cases at concentrations of 40 p. p. m., and usually lower concentrations were inhibitory, indicating that in general *C. circinans* was more sensitive than *P. brassicae* to allyl isothiocyanate (table 2).

TABLE 2.—*Germination of spores of Colletotrichum circinans in drops of allyl isothiocyanate solution*

Concentration (*p. p. m.*)	Germination [1]		
	Experiment 1	Experiment 2	Experiment 3
	Percent	*Percent*	*Percent*
0.0 [2]	70	59	
0.0 [3]	71	58	87
0.002	75	63	88
0.004	74	51	86
0.008	64	62	84
0.019	76	60	
0.039	66	57	82
0.078	75	56	78
0.156	70	58	74
0.312	74	58	80
0.625	71	43	82
1.25	65	59	80
2.5	40	33	38
5.0	6	26	2
10.0	2	0	0
20.0	3	0	0
40.0	0	0	0

[1] Figures represent the percent germination of 100 spores after 5 hours' incubation.
[2] Control prepared with distilled water only.
[3] Control and all mustard-oil solutions prepared in 1.0 percent ethyl alcohol.

EFFECTS OF MUSTARD OILS ON DISEASE DEVELOPMENT

The effect of mustard oils on infection and disease development as reported by Rochlin (*14, 15*) has already been mentioned. Comparative studies on the toxicity of the two oils on disease development were carried out as previously described on cabbage plants grown in the greenhouse.

Mustard-oil Injury to Cabbage

In addition to the toxic effects of the mustard oils to *Plasmodiophora brassicae*, it was found that considerable injury to the host resulted at certain concentrations. Typical injury is shown in figure 3. Affected plants exhibited symptoms typical of damping-off be-

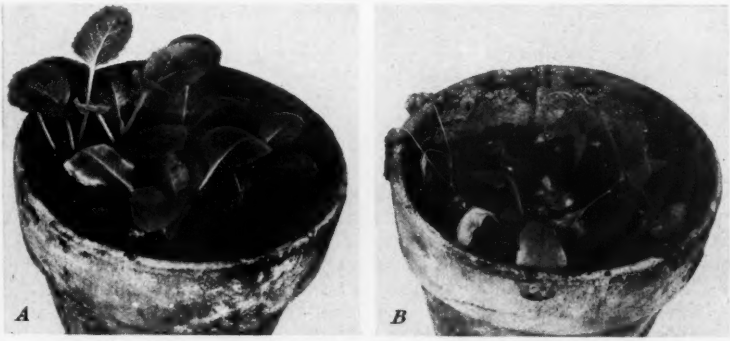

Figure 3.—Cabbage plants showing effect of beta-phenethyl isothiocyanate. *A*, plants transplanted with a liquid containing 300 p. p. m.; no injury resulted. *B*, plants transplanted with a liquid containing 400 p. p. m.; marked injury resulted.

cause of the rapid deterioration of the lower stem and subsequent collapse of the plant. Occasionally plants were able to develop roots above the point of injury and survive. Recovered plants could be readily recognized when removed from the soil by the complete absence of the main root or by the protrusion of the stump of the original root system and lower stem from a tuft of actively developing roots above the point of injury. When mixed with spore suspensions of *P. brassicae* the allyl oil caused some injury to cabbage at 100 p. p. m., while the phenethyl oil was toxic at 350 and 400 p. p. m.

Toxicity of Mustard Oils to Spores Used as Inoculum

The relative toxicity of the two mustard oils to the resting spores of *Plasmodiophora brassicae* was determined, infection and disease development of cabbage being used as an indication of spore inactivation. Spores were usually mixed with the oil 2 days before the suspension was used as inoculum; in experiments 1 and 5 (table 3) the exposure period was 3 and 4 days, respectively.

In certain instances, cabbage plants that had been protected from infection by the addition of oil to the inoculum showed greater vegetative growth than those severely diseased after inoculation with spore suspensions in oil solutions at subinhibitory concentrations. Dif-

TABLE 3.—*Relative toxicity of allyl and beta-phenethyl isothiocyanate when mixed with a spore suspension of Plasmodiophora brassicae used as inoculum*

Oil concentration (P. p. m.)	Percent of cabbage plants infected at concentrations (p. p. m.) indicated [1]											
	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5		Experiment 6	
	Allyl	Phenethyl	Allyl	Phenethyl	Allyl	Phenethyl	Allyl	Phenethyl	Allyl	Phenethyl	Allyl	Phenethyl
0.0	100	100	100	100	100	100	100	100	93	80	73	92
1.25	------	------	------	------	100	100	------	------	87	60	92	82
2.5	------	------	------	------	67	100	100	20	93	80	75	91
5.0	------	------	------	------	28	62	27	43	53	64	80	93
10.0	------	------	------	------	8	92	14	53	100	53	78	83
20.0	------	------	------	------	0	8	23	7	80	29	43	40
30.0	------	------	------	------	0	0	0	0	0	0	12	33
50.0	100	100	50	33	0	0	0	0	0	0	0	55
75.0	80	73	8	43	------	------	0	0	0	0	0	0
100.0	0	33	0	13	------	------	0	0	0	0	0	0
150.0	13	100	0	29	------	------	0	0	0	0	0	0
200.0	0	77	0	0	------	------	0	0	0	0	0	0
250.0	0	7	0	23	------	------	0	0	------	------	0	0
300.0	0	0	0	31	------	------	0	0	0	0	0	0
350.0	------	------	0	55	------	------	------	0	------	------	------	0
400.0	------	------	0	0	------	------	------	0	0	0	------	0

[1] 15 plants were inoculated with each suspension.

ferences of this nature are shown in figure 4. Infection was precluded by a transplanting solution containing 75 p. p. m. of allyl isothiocyanate (*B*); whereas 100 percent disease development took place, when a solution containing 50 p. p. m. was used (*A*).

The infectivity of the inoculum with and without removal of the oil at the end of the period of exposure was compared to determine whether the end results secured in table 3 were the effect in part of the carry-over influence of oil added with the suspension at the time of transplanting. It was possible to remove practically all of the oil by centrifugation. The results of three experiments are given in table 4.

FIGURE 4.—Effect on clubroot development of two concentrations of allyl isothiocyanate in spore suspensions used as transplanting liquids. *A*, at 50 p. p. m. infection was complete and disease severe. *B*, at 75 p. p. m. no infection occurred.

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TABLE 4.—*Relation of time of exposure of Plasmodiophora brassicae to mustard oils and the removal of oil from the inoculum, to the infectivity of the spore suspension*

Experiment No.	Oil used	Days exposed to oil	Oil present or absent in inoculum	Percent of cabbage plants infected at concentrations (p. p. m.) indicated [1]														
				0	1.25	2.5	5	10	20	30	50	75	100	150	200	250	300	350	400
1	Allyl	3	Present	100							100	80	0	13	0	0	0	-----	-----
			Absent	100							86	0	7	0	0	0	0	-----	-----
		10	Present	100							73	33	0	0	0	0	0	-----	-----
			Absent	100	-----	-----	-----	-----	-----	-----	100	58	8	36	23	47	14	-----	-----
		24	Present	100	-----	-----	-----	-----	-----	-----	93	20	0	0	0	0	0	-----	-----
			Absent	100	-----	-----	-----	-----	-----	-----	100	87	53	0	0	0	0	-----	-----
	Phenethyl	3	Present	100	-----	-----	-----	-----	-----	-----	100	73	33	100	77	7	0	-----	-----
			Absent	100	-----	-----	-----	-----	-----	-----	60	36	0	93	0	0	0	-----	-----
		10	Present	100	-----	-----	-----	-----	-----	-----	100	100	93	100	93	57	0	-----	-----
			Absent	100	-----	-----	-----	-----	-----	-----	100	100	100	93	36	8	0	-----	-----
		24	Present	100	-----	-----	-----	-----	-----	-----	100	47	60	100	0	0	0	-----	-----
			Absent	100	-----	-----	-----	-----	-----	-----	100	100	100	100	10	7	0	-----	-----
2	Allyl	2	Present	100	-----	-----	-----	-----	-----	-----	50	8	0	0	0	0	0	0	0
			Absent	93	-----	-----	-----	-----	-----	-----	29	45	71	33	0	22	31	0	7
		10	Present	83	-----	-----	-----	-----	-----	-----	0	0	0	0	0	0	0	0	0
			Absent	100	-----	-----	-----	-----	-----	-----	14	0	0	0	0	0	0	13	0
		23	Present	76	-----	-----	-----	-----	-----	-----	0	0	0	0	0	0	0	0	0
			Absent	93	-----	-----	-----	-----	-----	-----	0	0	0	0	0	0	0	0	0
	Phenethyl	2	Present	100	-----	-----	-----	-----	-----	-----	33	43	13	29	0	23	31	55	0
			Absent	93	-----	-----	-----	-----	-----	-----	6	46	27	86	85	23	7	31	0
		10	Present	55	-----	-----	-----	-----	-----	-----	0	7	0	0	0	0	0	0	0
			Absent	100	-----	-----	-----	-----	-----	-----	33	41	0	0	15	0	27	7	0
		23	Present	50	-----	-----	-----	-----	-----	-----	0	0	0	0	0	0	0	0	0
			Absent	100	-----	-----	-----	-----	-----	-----	0	0	0	0	0	0	0	0	0
5	Allyl	4	Present	93	87	93	53	100	80	0	0	0	0	0	0	-----	0	-----	0
			Absent	100	92	100	89	100	73	100	0	0	0	0	0	-----	0	-----	0
		14	Present	100	92	100	89	100	92	100	0	0	0	0	0	-----	0	-----	0
			Absent	100	100	86	100	100	90	93	0	0	0	0	0	-----	0	-----	0
	Phenethyl	4	Present	80	60	80	64	53	29	0	0	0	0	0	0	-----	0	-----	0
			Absent	100	93	100	100	100	73	14	0	0	7	0	0	-----	0	-----	0
		14	Present	90	90	100	100	100	100	0	0	0	9	0	0	-----	0	-----	0
			Absent	100	100	92	100	100	91	8	15	0	15	0	9	-----	12	-----	0

[1] 15 plants were inoculated with each suspension.

There was a tendency for the toxic effect of each oil to increase with length of exposure but it did not hold consistently with either one. There was also a tendency for the inoculum to be infective over a wider range of concentrations when the oil was removed before inoculation, indicating that at the end of a given exposure part of the spores were only inhibited and others killed. This trend, however, was not entirely consistent. It would seem, therefore, that the comparative effects given in table 3 are a fairly accurate index of relative toxicity of the oils and that changes in length of exposure and in removal of oil would not materially change the end result.

Relation of Spore Concentration to Toxicity

It has been shown by a number of investigators that toxic levels of chemicals may be considerably changed by different spore concentrations. Smith (*16*) reported that as the spore concentration increased in 4 percent phenol, the percentage of spores surviving exposure to the toxic substance increased. McCallan, Wellman, and Wilcoxon (*10*) observed that increasing the spore concentration in dilute copper sulfate solutions and in bordeaux mixture resulted in germination at higher concentrations of copper than was possible with lower spore concentrations. Pryor, Walker, and Stahmann (*13*) varied spore concentrations exposed to vapors of allyl isothiocyanate and observed that colony development was precluded by lower partial pressures of oil when the spore concentration had been reduced 100 times.

Trials were conducted to determine the effect of spore concentration of *P. brassicae* on the level of allyl isothiocyanate required to prevent infection in cabbage. The data (table 5) secured in three experiments showed that, in general, as the spore concentration increased from 25,000 to 800,000 spores per cubic millimeter the concentration of allyl isothiocyanate required to prevent infection progressively increased.

Table 5.—*Effect of increasing concentrations of Plasmodiophora brassicae spores on toxicity of allyl isothiocyanate*

Experiment No.	Spore concentration (spores per mm³.)	Percent of cabbage plants infected at concentrations (p. p. m.) indicated [1]												
		0.0	1.25	2.5	5.0	10.0	20.0	30.0	50.0	75.0	100	150	200	250	300
1	25,000	100	----	----	----	----	----	----	----	0	0	0	0	0	0
	50,000	----	----	----	----	----	----	----	----	0	0	0	0	0	0
	100,000	----	----	----	----	----	----	----	----	79	0	0	0	0	0
	200,000	71	----	----	----	----	----	----	----	100	36	0	0	0	0
4	25,000	100	----	100	27	14	23	0	0	0	0	0	0	0	0
	50,000	97	----	73	40	40	58	7	0	0	0	0	0	0	0
	100,000	100	----	100	79	80	27	0	0	0	0	0	0	0	0
	200,000	100	----	100	93	64	8	0	0	0	0	0	0	0	0
6	25,000	73	92	75	80	79	43	12	0	0	0	0	0	0	0
	100,000	75	75	90	100	57	64	50	44	29	0	0	0	0	0
	200,000	75	90	86	82	100	100	100	71	37	0	0	0	0	0
	400,000	100	92	73	80	91	53	50	22	20	25	0	0	0	0
	800,000	86	87	71	80	100	77	64	62	33	50	0	0	0	0

[1] 15 plants inoculated with each suspension.

It has been reported above that at certain levels the oil in the spore suspension was toxic to the host plant. As the concentration of spores increased, the concentration of oil required to cause death of the host plant progressively increased. This is illustrated in figure

5. In table 6 are given the number of surviving plants at various concentrations of oil and spores. It is apparent that at each oil level at which plant injury occurred the toxicity was usually reduced with increase in spore concentration. Thus it is evident that the spores take up the oil or detoxify it in proportion to their number per given volume of suspension. Consequently, the concentration of spores in the inoculum is important in determining whether or not a given level of oil will be sufficiently toxic to prevent infection or to cause host injury.

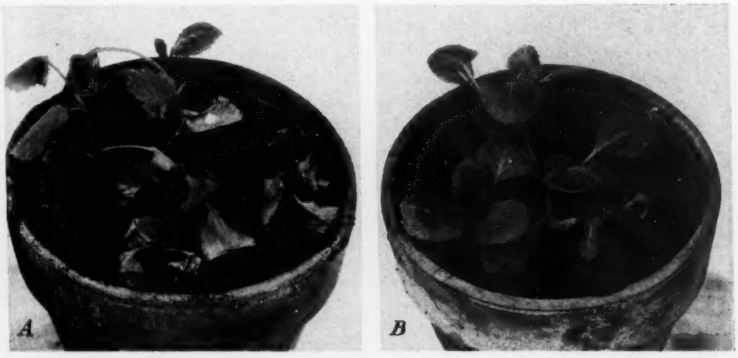

FIGURE 5.—Influence of spore concentration on the phytotoxic effect of allyl isothiocyanate in the inoculum. *A*, plants inoculated with a suspension containing 50,000 spores per cubic millimeter and 200 p. p. m. of oil; injury severe. *B*, Plants inoculated with a suspension containing the same concentration of oil but 100,000 spores per cubic millimeter; injury slight.

TABLE 6.—*Effect of increasing concentrations of Plasmodiophora brassicae spores on the survival of cabbage plants at various levels of allyl isothiocyanate*

Oil concentration p.p.m.	Number of plants surviving from 15 planted at number of spores per mm.[3] indicated—			
	25,000	50,000	100,000	200,000
0	14			14
75	12	14	14	15
100	12	12	14	14
150	0	12	10	13
200	3	6	9	13
250	2	5	4	7
300	0	0	8	5
350	0	0	0	1
400	0	0	0	1

EFFECTS OF MUSTARD OILS ON CLUB SIZE

It was noted in certain of the toxicity experiments described above that the clubs tended to be larger in plants exposed to subinhibitory oil concentrations containing spore suspensions than in plants developing in the absence of oil. Clubs were weighed in each trial in order to determine the effects of oils on club size. It became apparent that mustard-oil solutions at subinhibitory concentrations were often capable of favoring club development by protecting the infected

root from secondary decay. Since in practically all trials, clubs developing in the absence of oils showed a greater tendency to rot than those exposed to oils, the influence of secondary organisms in the development of the club was a factor in club size. However, final evaluation of the data failed to show that club size was increased by the presence of mustard oil in the inoculum.

DISCUSSION

Allyl and beta-phenethyl isothiocyanates have been shown to be highly toxic to a number of fungi (*7*, *13*, *19*). The former occurs in the roots of horseradish, while the latter is present in the roots of horseradish, cabbage, black mustard, white mustard, and turnip (*17*). In the first case, and presumably in the second, they occur as glucosides rather than as free oils. The glucoside of the allyl oil, sinigrin, has relatively little or no toxicity to fungi tested (*19*). The possible relation of the mustard oils to resistance in certain varieties of mustard and turnip to clubroot has been the occasion of the present study of their effect upon the pathogen, *Plasmodiophora brassicae*.

The two oils were compared by adding them in various concentrations to spore suspensions of the pathogen. When the relative number of zoospores per microscopic field was used as a criterion and when the oils were compared on a molecular basis, the phenethyl oil was generally more toxic than the allyl oil. On the other hand, when the spore suspensions so treated were used as inocula around the roots of young cabbage transplants, the percentage of diseased plants indicated that the two oils were usually about equal in effect and that sometimes the allyl oil was the more toxic. When the time of exposure was increased and when the oil was removed at the end of the exposure period, no consistent change in results was observed.

Both oils stimulated spore germination of *P. brassicae* at levels below the inhibitory concentrations, although allyl isothiocyanate did so more consistently. No stimulation of spore germination of *C. circinans* was effected by allyl isothiocyanate. It is possible that the oils are specific to *P. brassicae* in their sitmulatory properties. Stimulation of fungi by toxic substances has been reported by a number of investigators. Latham (*8*) showed that the vapors of chloroform stimulated mycelial growth of *Sterigmatocystis*. Duggar (*5*) reported that ethyl alcohol and copper sulfate in low concentrations stimulated germination of certain fungus spores. Brown (*2*) showed that volatile substances arising from bruised leaves and apple tissue stimulated germination of *Botrytis* spores, whereas substances from potato tissue, orange rind, onion leaves, and bulb scales inhibited spore germination. Brown (*1*) showed that substances liberated from plant leaves and petals into drops of water are capable of either stimulating or inhibiting germination.

The levels of allyl isothiocyanate preventing infection and disease development were shown to be influenced by variation in spore concentration. These observations agree with results reported with various fungi by Smith (*16*), McCallan, Wellman, and Wilcoxon (*10*), and Pryor, Walker, and Stahmann (*13*).

Considerable variation between experiments was encountered throughout the investigation. Although the exact reason for this

lack of uniformity has not been determined, it is obvious that a number of factors are concerned. Among these are the effect on the oils of secondary organisms in the root extracts; variations within the clubroot inoculum from different locations; variation in period of storage of clubbed roots; and variations in the greenhouse environment influencing infection and disease development. McCallan and Wilcoxon (*9*), who were able to grow their test organisms in pure culture under constant conditions, found that spores from replicate transfers varied a great deal more than would be expected from random sampling and they suggest that spores from the same tube be used in laboratory tests of fungicides in order to eliminate the error arising from the use of replicate transfers. The differences existing between different spore samples in the tests reported in this paper are undoubtedly of sufficient magnitude to account for a large part of the variability in results.

Evidence concerning the lack of importance of mustard oils in resistance to *P. brassicae* has been obtained through three different lines of approach. Pryor (*12*) showed that even though the mustard-oil content of resistant and susceptible strains of turnip and black mustard and of susceptible strains of cabbage was definitely reduced by low-sulfur nutrition, the inherent disease reaction was little altered. He concluded that the sulfur oils are not essential in enabling the host to prevent or retard clubroot development in its tissues. Stahmann, Link, and Walker (*17*) determined the mustard-oil content as well as the myrosin activity in the tissues of resistant and susceptible strains of turnip and black mustard and were unable to correlate either of these properties with resistance. In this paper it has been shown that the mustard oils, allyl and beta-phenethyl isothiocyanates, are very toxic to the clubroot organism at concentrations as low as 10 p. p. m. of the free oils. It was estimated that the beta-phenethyl oil occurred in turnip and mustard roots at concentrations ranging from 0.010 to 0.024 percent (100 to 240 p. p. m.). If the oils occurred in the free state they should be sufficiently concentrated to prevent infection. However, it has not been shown that free oil is liberated from the roots into the soil solution as has been reported for germinating cruciferous seeds by Denny (*4*), and if such were the case it seems unlikely that oil concentrations comparable to those combined as glucosides within the tissue could be maintained outside the plant. There is still no adequate explanation of resistance since chemical analyses (*17*) show about equal amounts of the oils in roots of very susceptible species such as cabbage and in both resistant and susceptible turnips and black mustard. No evidence of differential enzyme activity was found which might control the release of free oil in resistant as compared with susceptible plants (*17*).

It is evident both from these investigations and from earlier ones (*17*) that the mere presence in the host of a material highly toxic to one of its pathogens is no proof that the toxic substance functions in restriction of pathogenicity. The data presented herein would indicate quite as strongly that these oils liberated in minute amounts might even be stimulatory to *P. brassicae* and enhance infection rather than restrict it. In fact there may be some connection between the specific stimulatory effect of these oils to *P. brassicae* and the fact that its obligate parasitism is restricted to the *Cruciferae*.

SUMMARY

Direct observations on the germination of resting spores of *Plasmodiophora brassicae* Wor. exposed to allyl and beta-phenethyl isothiocyanate were made. Either oil in certain concentrations is capable of inhibiting spore germination. Little difference in the toxicity of the two oils was observed when compared on a weight basis, but when compared on a molecular basis the phenethyl oil was almost twice as toxic as allyl isothiocyanate.

When the toxicity of the oils to the clubroot organism in spore suspensions was tested by inoculation of cabbage plants, the two were most often approximately equal in toxicity, when compared on a molecular basis, although in certain instances allyl isothiocyanate was definitely more toxic than phenethyl isothiocyanate. Increasing the time of exposure of the spores to oil before planting, sometimes but not consistently resulted in an increased toxic effect of the oil. In some experiments removal of oil from the inoculum at the end of the exposure period indicated that the oil had had an inhibitive effect on some spores and a lethal effect on others.

At concentrations below the toxic level, both oils were definitely stimulatory to germination of spores, but the allyl oil stimulated germination more consistently than the beta-phenethyl isothiocyanate. The spores of *Colletotrichum circinans* (Berk.) Vogl. were not stimulated in germination by allyl isothiocyanate under the experimental conditions employed.

As the concentration of spores was increased, higher concentrations of allyl isothiocyanate were required to prevent disease development.

No consistent change in relative club size resulted from the presence of mustard oils in the spore inoculum or from exposure of spores to oil before their use as inoculum, except that at sublethal levels there was a tendency for the oil to protect the club from decay by secondary soil organisms.

Concentrations of potentially available beta-phenethyl isothiocyanate in the roots of both the resistant and susceptible crucifers examined are roughly comparable to the concentrations of oil required to prevent disease development and spore germination when mixed with spore suspensions of *P. brassicae*. However, the liberation of this oil in appreciable quantities from the roots of cruciferous plants has not been demonstrated. If it may be assumed that mustard oils are liberated from roots of crucifers into the soil solution, it seems unlikely that oil concentrations comparable to those combined as glucosides within the tissue could be maintained outside the plant. Furthermore, from what is known of the concentration of the phenethyl oil in cruciferous roots, as great protection would be expected to be afforded the susceptible varieties of turnip and mustard as is afforded the resistant varieties, since the oil content of the roots of the susceptible and resistant varieties is about the same. If oil concentrations in the soil solution outside the plant were below the toxic level, a stimulatory effect on *P. brassicae* spores could conceivably be induced. In fact it is possible that the mustard oils, if present in the proper concentration in the soil solution, might actually predispose cruciferous plants to disease rather than increase their resistance.

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RESISTANCE OF TOBACCO TO BLUE MOLD (PERONOSPORA TABACINA)[1]

By E. E. CLAYTON [2]

Senior pathologist, Division of Tobacco Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

All varieties of tobacco (*Nicotiana tabacum* L.) grown in the United States are highly susceptible to the blue mold disease, caused by *Peronospora tabacina* Adam. Therefore, to provide a sound basis for a breeding program, a search has been made for adequate resistance to blue mold in the genus *Nicotiana*. The available material consisted of some 1,000 collections of *N. tabacum* from all parts of the world, but in particular from Mexico and Central and South America.[3] In addition, seed was available [4] from many of the wild *Nicotiana* species. The work was begun in 1933 and has been continued without interruption to the present time.

MATERIALS AND METHODS

The procedure first followed in testing for resistance to blue mold was to sow the seeds in rows (fig. 1) and expose the plants when 5 or 6 weeks old to blue mold attack. However, it was soon discovered that age and vigor of growth had a very marked effect on the susceptibility of plants to blue mold. Hence, to obtain more uniform material, the seedlings were transplanted into 2½-inch earthen pots, and great care was taken to provide optimum conditions for rapid growth. The importance of uniformly severe disease attacks was also apparent. Thus, genotypes that endured moderate blue mold attacks without serious damage might be completely killed by severe epidemics. It was impossible to provide uniformly severe conditions in plant beds; so, although plant-bed tests were used extensively, major dependence was placed on greenhouse experiments. Thus, it was found that from November to March quite uniformly severe conditions could be provided in the greenhouse, and that when potted plants 6 to 8 weeks old were used, the results corresponded very closely with those obtained in plant-bed tests under the severest conditions. The usual number of greenhouse plants used per lot was 10, and conclusions regarding susceptibility of *Nicotiana* species were based on at least 5 separate tests conducted at intervals over a period of several years. Wherever the need for maximum accuracy was apparent, as, for example, with the most resistant species and

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[2] The writer acknowledges the very competent assistance of H. H. Foster and C. C. Yeager, formerly of the Division of Tobacco Investigations.

[3] These collections were made by the Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering.

[4] Largely through the courtesy of the University of California Botanical Garden.

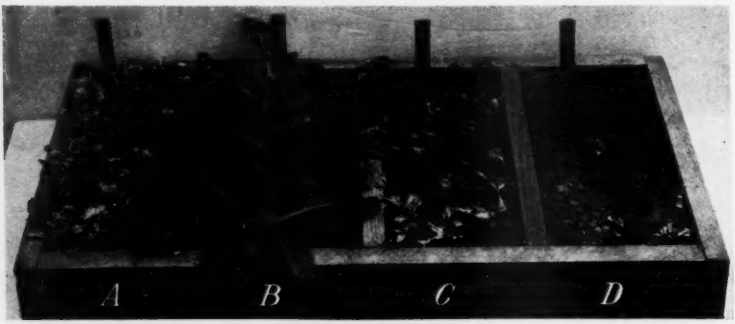

FIGURE 1.—Plants showing response of different species of *Nicotiana* to blue mold attack. *A* and *C*, Varieties of *Nicotiana tabacum; D, N. sylvestris;* all three susceptible to blue mold. *B, N. debneyi,* highly resistant. Photographed in 1934; at that time *N. debneyi* was regarded as a strain of *N. suaveolens* Lehm.

certain slightly resistant genotypes of *N. tabacum,* testing was much more extensive and prolonged. The recording of disease data was facilitated by the use of index numbers as shown in table 1.

TABLE 1.—*Index numbers used to record the amount of blue mold disease and the degree of resistance of Nicotiana species*

Index number	Amount of disease	Degree of resistance
0	No evidence of disease	Immune.
1	Small lesions; no visible sporulation	Highly resistant.
2	Moderate-size lesions; slight sporulation	Moderately resistant.
3	Large lesions and partial defoliation; abundant sporulation	Slightly resistant.
4	General defoliation; abundant sporulation; plants killed	Susceptible.

RESULTS

RESISTANCE IN NICOTIANA TABACUM

Search for resistance to blue mold in *Nicotiana tabacum* resulted in the discovery of a number of South American types, chiefly from Argentina, that were slightly resistant. A good representative of this group was T. I. 57. Under conditions of moderate attacks of blue mold in the plant bed, this genotype frequently showed striking evidences of resistance. However, when disease conditions were severe, resistance broke down almost completely. Subsequently, T. I. 57 was crossed with two flue-cured varieties, Gold Dollar and White Stem Orinoco, and large F_2 populations were grown and tested. Plants surviving severe attacks of blue mold were tested as F_3's. However, despite extensive work over a 3-year period, the full resistance of the T. I. 57 parent was never completely recovered. Evidently, resistance was dependent on a number of genes; consequently, it seems that there is little prospect of obtaining satisfactory resistance to blue mold in *N. tabacum* by intraspecific hybridization.

RESISTANCE OF VARIOUS SPECIES OF NICOTIANA

By means of the methods outlined, the comparative resistance of some 33 species of *Nicotiana* has been determined under what might

be termed plant-bed conditions as they prevail in the United States. This, as indicated, has involved tests in actual beds at the Coastal Plain Experiment Station, Tifton, Ga.; the Pee Dee Experiment Station, Florence, S. C.; the McCullers and Oxford branch stations, North Carolina; and the Arlington Experiment Farm, Arlington, Va.; and repeated tests in the greenhouse with transplanted seedlings 6 to 8 weeks old. Except for a number of species tested only a few times, the results are shown in table 2.

TABLE 2.—*Comparative resistance of Nicotiana species to blue mold under plant-bed conditions in the United States*

Group 0–1, immune to highly resistant	Group 2, moderately resistant	Group 3, slightly resistant	Group 4, susceptible
N. debneyi Domin.[1]	*N. acuminata* (Grah.) Hook.	*N. alata* Link and Otto.	*N. benthamiana* Domin.[1]
N. exigua H.-M. Wheeler.[1]	*N. caesia* Suksd.	*N. attenuata* S. Wats.	*N. covanillesii* Dun.
N. goodspeedii H.-M. Wheeler.[1]	*N. gossei* Domin.[1]	*N. langsdorffii* Weinm.	*N. glauca* Grah.
N. longiflora Cav.	*N. paniculata* L.	*N. otophora* Griseb.	*N. glutinosa* L.
N. maritima H.-M. Wheeler.[1]	*N. rustica* L.	*N. quadrivalvis* Pursh.[2]	*N. nesophila* Johnst.
N. megalosiphon Heurck and Muell.-Arg.[1]		*N. raimondii* Macbride.	*N. nudicaulis* S. Wats.
N. plumbaginifolia Viv.		*N. repanda* Lehm.	*N. stocktoni* Brandeg.
N. rotundifolia Lindl.[1]		*N. sanderae* W. Wats.	*N. tabacum* L.
		N. sylvestris Speg. and Comes.	*N. trigonophylla* Dun.
		N. tomentosa Ruiz and Pavon.	*N. wigandioides* Koch and Fint.

[1] Australian species; all other species are of American origin.
[2] This species has commonly been called *Nicotiana bigelovii* S. Wats.

For convenient consideration, the 33 species represented in table 2 have been divided into 4 groups on the basis of their resistance to blue mold, but actually they represented a complete gradation from highly susceptible to immune. The 8 species listed in group 0–1 showed no evidence of disease in any plant-bed test and hence might be listed as immune. Group 2 (moderately resistant) showed only a slight amount of disease under plant-bed conditions. Group 3 (slightly resistant) was a disease-tolerant group. The plants developed abundant infection but were rarely defoliated completely and usually recovered. Group 4 (susceptible) requires no comment other than to mention that *Nicotiana benthamiana*, *N. nesophila*, and *N. stocktoni* were more susceptible than the others and could have been designated as a separate "very susceptible" group.

As table 2 indicates, the Australian species on the whole were more resistant to blue mold than the American species. Of the 8 Australian species, 6 were highly resistant to immune; 1 was moderately resistant; and 1 (*Nicotiana benthamiana*) was highly susceptible. Wheeler (*8*) [5] lists *N. benthamiana* as native to the northwest coast of Australia, where blue mold presumably does not occur. Of the 25 American species, only 2 (*N. longiflora* and *N. plumbaginifolia*) have been rated as immune to highly resistant.

Reference has already been made to the effect of plant age on resistance to blue mold. Even plants of the susceptible species become more resistant as they grow older, and the plants of some species pass through a complete transformation from highly susceptible when very young to completely immune a few weeks later (fig. 2).

[5] Italic numbers in parentheses refer to Literature Cited, p. 87.

The resistance ratings in table 2 are based on severe plant-bed conditions and compare well with the reaction of greenhouse plants 6 to 8 weeks old. It is of much interest to know the reaction of younger plants of the species in the immune to highly resistant group of table 2. To obtain such data, seedlings were grown under uniform conditions and inoculated when they were 14, 24, and 35 days old. Since it was not feasible to transplant these very small plants, the seed was sown in pots and the plants were thinned to uniform stands. Experiments 1, 2, and 3, the data for which are shown in table 3, were made at widely separated intervals with three entirely separate crops of plants.

TABLE 3.—*Resistance to blue mold of Nicotiana plants 14, 24, and 35 days old*

Nicotiana species	Age of plants	Leaves sporulating				*Nicotiana* species	Age of plants	Leaves sporulating			
		Experiment No.—			Mean			Experiment No.—			Mean
		1	2	3				1	2	3	
	Days	*No.*	*No.*	*No.*	*No.*		*Days*	*No.*	*No.*	*No.*	*No.*
N. tabacum	14	65	48	50	54.3	*N. plumbaginifolia*	14	38	15	11	21.3
	24	85	96	44	75.0		24	2	8	12	7.3
	35	130	104	42	92.0		35	0	8	2	3.3
N. glauca	14	50	54	48	50.7	*N. debneyi*	14	21	8	10	13.0
	24	76	52	67	65.0		24	0	8	5	4.3
	35	135	32	128	98.3		35	0	0	0	0
N. rustica var. *humilis* Schrank.	14	98	24	28	50.0	*N. rotundifolia*	14	0	14	7	7.0
	24	103	11	11	41.7		24	0	3	4	2.3
	35	60	8	24	30.7		35	0	0	0	0
N. rustica var. *brasilia* Schrank.	14	68	5	4	25.7	*N. maritima*	14	18	4	8	10.0
	24	6	11	0	5.7		24	0	3	14	5.7
	35	4	10	14	9.3		35	0	0	0	0
N. paniculata	14	60	23	43	42.0	*N. megalosiphon*	14	0	4	12	5.3
	24	42	44	6	30.7		24	0	3	0	1.0
	35	8	0	1	3.0		35	0	0	0	0
N. caesia	14	14	11	18	14.3	*N. goodspeedii*	14	2	4	0	2.0
	24	8	6	8	7.3		24	0	0	0	0
	35	6	11	8	8.3		35	0	0	0	0
N. longiflora	14	80	12	62	51.3	*N. exigua*	14	0	0	0	0
	24	24	12	34	23.3		24	0	0	0	0
	35	3	4	0	2.3		35	0	0	0	0

The species listed in table 3 include two that were rated as susceptible in table 2—*Nicotiana tabacum* and *N. glauca*; three that were moderately resistant—*N. rustica*, *N. paniculata*, and *N. caesia*; and all eight of the immune to highly resistant group. The amount of sporulation is the best available measure of plant resistance in tests of this sort; hence, the data in table 3 are given in terms of the number of sporulating leaves. It should be noted that in some species the actual number of leaves exposed was two or three times as many with plants 35 days old as with plants 14 days old. With a susceptible species such as *N. tabacum*, the increase in resistance as the plants grow is slow, and hence the larger number of leaves exposed with 35-day plants was usually reflected in a larger number of sporulating leaves. However, with the moderately resistant species *N. rustica* and *N. paniculata* this was not true. By the time these plants were 35 days old they had developed sufficient resistance to reduce greatly the actual number of sporulating leaves. *N. caesia* differed from any

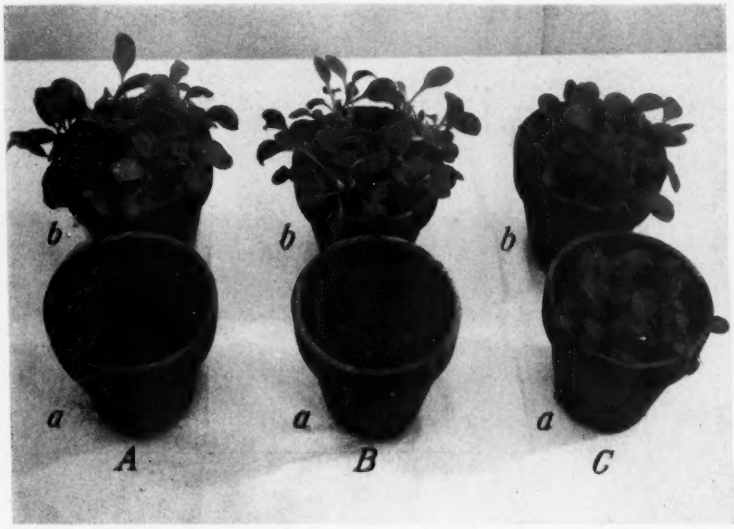

FIGURE 2.—Tobacco plants showing relation of age to susceptibility to blue mold. Each pot originally contained 25 plants; all were sown at the same time. *A*, *Nicotiana rustica: a*, Plants inoculated when 14 days old, susceptible and killed; *b*, plants inoculated when 35 days old, moderately resistant, disease limited to leaf lesions. *B*, *N. maritima: a*, Plants inoculated when 14 days old, completely susceptible; *b*, plants inoculated when 35 days old, immune. *C*, *N. exigua: a*, Plants inoculated when 14 days old; *b*, plants inoculated when 35 days old; both groups immune.

other species in that, while it was quite resistant at the start, resistance increased very little as the plants grew older.

Reference to table 3 shows that the difference between species such as *Nicotiana rustica*, *N. paniculata*, and *N. caesia*, which are in the moderately resistant group in table 2, and species such as *N. longiflora* and *N. plumbaginifolia*, which are in the immune to highly resistant group, is purely one of degree. As indicated earlier, resistant species showing no blue mold in any actual plant-bed test were placed in group 0–1 of table 2; but the results given in table 3 indicate that two of these, *N. longiflora* and *N. plumbaginifolia*, are really only a little more resistant than certain species classed as moderately resistant in table 2.

Nicotiana debneyi, *N. rotundifolia*, *N. maritima*, *N. megalosiphon*, *N. goodspeedii*, and *N. exigua*, as indicated in table 3, make a compact group of highly resistant species, although, if plants only 14 days old are inoculated, the first three of this group may show considerable leaf infection. To bring out this relation of plant age to susceptibility to blue mold still more clearly, an experiment was made in which the number of leaves exposed was counted so that data could be given on the basis of percentage of leaves sporulating. The percentage of plants killed was also noted (table 4).

TABLE 4.—*Relation of age of Nicotiana plants to their susceptibility to blue mold*

Nicotiana species	Age of plants	Leaves[1] sporulating	Plants[2] killed	*Nicotiana* species	Age of plants	Leaves[1] sporulating	Plants[2] killed
	Days	*Percent*	*Percent*		*Days*	*Percent*	*Percent*
N. tabacum var. Gold Dollar	15	100	100	*N. longiflora*	15	88	64
	25	100	100		25	30	0
	35	100	100		35	24	0
N. tabacum var. Maryland Broadleaf	15	97	96	*N. plumbaginifolia*	15	42	24
	25	100	100		25	2	0
	35	100	100		35	0	0
N. glauca	15	100	100	*N. debneyi*	15	32	28
	25	100	100		25	0	0
	35	100	100		35	0	0
N. alata	15	100	100	*N. rotundifolia*	15	0	0
	25	100	80		25	0	0
	35	100	76		35	0	0
N. rustica var. *humilis*	15	98	96	*N. megalosiphon*	15	0	0
	25	89	60		25	0	0
	35	60	0		35	0	0
N. rustica var. *brasilia*	15	75	44	*N. exigua*	15	0	0
	25	6	0		25	0	0
	35	3	0		35	0	0
N. paniculata	15	90	84				
	25	42	44				
	35	38	0				

[1] The number of leaves exposed averaged 100.
[2] The number of plants exposed averaged 25.

The data in table 4 show that in this experiment plants from 15 to 35 days old were equally susceptible in the species *Nicotiana tabacum* and *N. glauca* and equally resistant in the species *N. rotundifolia*, *N. megalosiphon*, and *N. exigua*; but in all species intermediate between these groups, susceptibility varied widely, depending on plant age. The results in table 4 suggest that *N. plumbaginifolia* was distinctly more resistant than *N. longiflora*; but, as indicated in table 3, the difference usually was not great, and both species reacted as highly resistant to immune by the time they were 6 to 7 weeks old. It is quite apparent, however, that anyone making resistance tests with very young *N. longiflora* seedlings would list this species as highly susceptible. *N. paniculata* and *N. rustica* also were very susceptible when young but usually became highly resistant by the time they were exposed to attack in the plant beds.

CROSSES BETWEEN NICOTIANA TABACUM AND RESISTANT SPECIES

Since the object of this work was to provide a foundation for the breeding of satisfactory blue mold resistant varieties of *Nicotiana tabacum*, the question arises as to whether *N. tabacum* can be successfully crossed with any of the group of highly resistant to immune *Nicotiana* species listed in table 2. The reports of Malloch and Malloch (*4*), Pal (*6*), Kostoff (*3*), and others are unanimous in concluding that self-fertile or cross-fertile hybrids have never been obtained between *N. tabacum* and any of these species. The recent development of colchicine naturally suggested its use, but extensive colchicine treatments on the seed and plants of these hybrids have given no results of positive value. Other methods, however, have gradually been developed that are more successful, and work is now in progress with progeny from *N. tabacum* crossed with *N. megalosiphon*, *N. debneyi*, *N. longiflora*, and *N. plumbaginifolia*. The results of this work suggest that it may be possible to use the very high degree of blue mold resistance found in certain wild *Nicotiana* species in breeding resistant va-

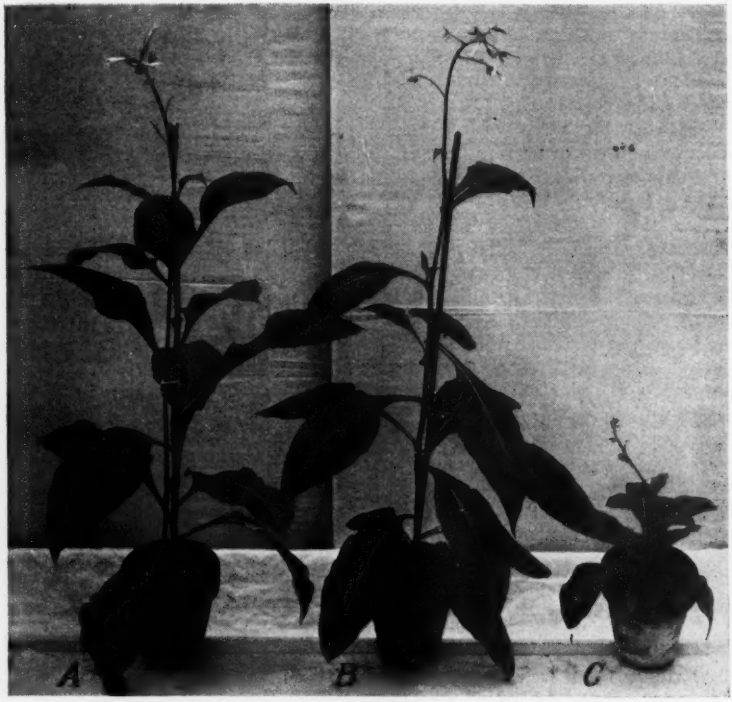

FIGURE 3.—Hybrid (*B*) produced by crossing blue mold resistant *Nicotiana debneyi* (*C*) with blue mold susceptible *N. tabacum* (*A*).

rieties of the cultivated *N. tabacum*. The undertaking is one of extreme difficulty, however, and the progress here reported represents merely the first step in its accomplishment (fig. 3).

DISCUSSION

Many investigators have reported briefly on the resistance of *Nicotiana* species to blue mold, but the reports are quite confusing. Thus, Angell and Hill (*2*) concluded that none of the *Nicotiana* species tested showed appreciable resistance to blue mold and hence that no promising basis for a program of breeding for disease resistance existed. On the other hand, Smith-White et al. (*7*) concluded that a high degree of blue mold resistance was available in the species, and they listed *Nicotiana debneyi* as immune. The writer has included *N. debneyi* among the group of species immune under plant-bed conditions in the United States (table 2). However, as indicated in tables 3 and 4, the 14-day-old seedlings of *N. debneyi* are quite susceptible to blue mold. Hence, depending on the age of the plants inoculated, *N. debneyi* might be reported as either susceptible or immune, and this situation obtains with other *Nicotiana* species. Therefore, unless information is available as to the age of the plants inoculated, data on susceptibility or resistance to blue mold are likely to

be misleading. In the reports on blue mold resistance already published, no mention has been made of this age factor; hence the basis for contradictory conclusions is evident.

In addition to age, there are other factors that tend to promote conflicting reports on blue mold resistance. One is the severity of disease attack. Certain *Nicotiana tabacum* genotypes may appear quite resistant under conditions of mild disease attack. The data in table 3 indicate the marked variability that can be expected in different tests. Host vigor has a marked effect on susceptibility. When slow-growing, tough tobacco plants and fast-growing, tender plants are equally exposed to blue mold, the former are scarcely affected, whereas the latter are defoliated. Another source of confusion has been the clouded taxonomic condition of the genus *Nicotiana*. Thus, Angell and Hill (*2*) referred to Adam (*1*) as finding that *N. longiflora* was susceptible to blue mold. However, inquiry reveals that the species tested by Adam was probably *N. suaveolens* var. *longiflora* Benth., which is now called *N. megalosiphon*. This is entirely different from the present *N. longiflora*, which is a South American species and does not occur in Australia.

A final source of variability in reports on species resistance, and one that may be of great importance, is the existence within a species of genotypes differing greatly in their resistance to blue mold. A distinct difference of this sort is shown in table 3 between *Nicotiana rustica* varieties *brasilia* and *humilis*. In these experiments, two collections of *N. longiflora* were used that were morphologically similar. However, this species is known to be very polymorphic, and Millán (*5*) listed four separate varieties differing greatly in appearance. Consequently it is quite possible that *N. longiflora* genotypes exist that differ greatly in their blue mold reaction from the ones tested. Therefore any conclusion as to species resistance can apply only to the particular material tested, and a complete picture could be secured only by testing not one or two but many collections of each species. The object of the present study was to establish a definite basis for a sound program of breeding for blue mold resistance. It is believed that this object has been achieved, because the resistance of any of the eight species making up group 0–1 of table 2 would be entirely adequate from the point of view of practical blue mold control in the United States.

SUMMARY

Tests with over 1,000 collections of *Nicotiana tabacum* have shown that none possessed resistance to blue mold adequate to provide a basis for a breeding program.

Tests with other species of *Nicotiana* have shown that many are highly resistant to blue mold but that the expression of resistance is complicated by a number of factors. The age of the plants tested was the most important single factor, and by exposing greenhouse-grown plants to blue mold when they were 6 to 8 weeks old results were obtained that compared closely with those obtained under the most severe out-of-door plant-bed conditions.

The age at which plants of different species became highly resistant or immune to blue mold was 6 to 7 weeks for *Nicotiana longiflora* and *N. plumbaginifolia*; 3 to 4 weeks for *N. debneyi*, *N. rotundifolia*, *N. maritima*, and *N. megalosiphon;* and 2 weeks for *N. goodspeedii*. *N.*

exigua was immune at all stages of growth. Under out-of-door plant-bed conditions no infection was observed on any of these eight species.

Successful crosses have been made between *Nicotiana tabacum* and *N. debneyi, N. megalosiphon, N. longiflora, and N. plumbaginifolia.*

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COMPOSITION AND DIGESTIBLE NUTRIENT CONTENT OF NAPIER GRASS LEAVES[1]

By R. W. KIDDER[2]

Assistant animal husbandman,
Everglades Branch Station, Florida Agricultural Experiment Station

INTRODUCTION

Napier grass (*Pennisetum purpureum* Schum.), also known as elephant grass and Merker grass, is a rank-growing perennial which is being used extensively for rotational pastures, as silage, and as a soil-improving crop, in Florida, Hawaii (*10*, *11*),[3] Trinidad (*8*, *9*), and other tropical and semitropical regions. This paper reports the results of a study to determine the composition and digestible nutrients of leaves of Napier grass harvested in a way to simulate grazing by cattle.

REVIEW OF LITERATURE

No analyses of Napier grass leaves or of that part of the plant consumed by cattle in natural grazing have been found in the literature. Wilsie, Akamine, and Takahashi (*10*) reported the composition of the "palatable portion" of the plant harvested at varying growth intervals which they designated as "all leaves and that part of the plant above and including the fifth visible ligule from the tip of the culm." Paterson (*8*, *9*) reported the composition of two varieties, for the entire plant, harvested at short intervals.

Harrison (*4*) conducted digestion trials with fresh Napier grass and found the digestion coefficients to be: For crude protein 63, for crude fiber 64, for nitrogen-free extract 60, and for crude fat 57. The chemical composition and digestibility of Napier grass as reported in the literature are summarized in table 1.

The digestion coefficients for Napier grass silage were determined by Neal, Becker, and Arnold (*7*) to be: For crude protein 29, for crude fiber 50, for nitrogen-free extract 40, and for crude fat 65. Digestibility experiments have been reported on Napier grass by Carbery, Chatterjee, and Talapatra (*2*), but as theirs was a study of mineral balances no digestion coefficients were included in the report.

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[2] The writer acknowledges his indebtedness to Director H. P. Rusk and Dr. H. H. Mitchell, of the Illinois Agricultural Experiment Station, for assistance and advice in connection with the original thesis; to Dr. W. M. Neal, formerly of the Florida Agricultural Experiment Station, for supervision of the digestion trial; to Dr. L. L. Rusoff, now of the Louisiana Experiment Station, for making most of the chemical analyses; and to Roy E. Blaser, Dr. W. G. Kirk, and P. T. Dix Arnold, of the Florida Station, for permitting the use of 23 analyses of Napier grass taken during 2 years of rotational grazing trials with beef and dairy cattle.

[3] Italic numbers in parentheses refer to Literature Cited, p. 92.

EXPERIMENTAL METHODS

The digestion trial, carried out at Gainesville, Fla., was conducted according to the method outlined by Forbes and Grindley (*3*). The Napier grass consisted of the leaves with the tender-growth tips plucked by hand twice daily in a manner to simulate grazing, from plots representing the growth of the preceding 4 weeks, the plots having been harvested in the same manner at that time. This freshly cut Napier grass constituted the entire nutrient intake of four mature Jersey steers for 4 consecutive 5-day experimental periods, following a 10-day preliminary feeding period. The feces were collected manually and stored in galvanized-iron cans. Samples were taken daily for dry-matter analyses and in triplicate for nitrogen determinations on the fresh material. Dry-matter samples were composited by 5-day periods for proximate analyses.

TABLE 1.—*Composition and digestibility of Napier grass as reported by several investigators*

Growth period (weeks)	Digestion trial No.	Composition on dry basis						Total digestible nutrients
		Dry matter	Crude protein	Crude fiber	Nitrogen free extract	Crude fat	Ash	
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
WILSIE, AKAMINE, AND TAKAHASHI (*10*)								
6			7.9	28.8	41.9	2.2	19.2	
8			6.9	30.7	41.2	2.0	19.2	
10			6.0	32.0	42.5	1.9	17.6	
12			5.9	32.6	42.4	2.0	17.1	
14			5.6	33.9	43.1	2.0	15.4	
PATERSON (*8*)								
4		14.5	9.9	24.0	[1] 47.3		18.8	
8		16.5	7.4	27.4	[1] 48.4		16.8	
12		20.7	6.1	31.5	[1] 45.7		16.7	
PATERSON (*9*)								
2		13.3	11.7					
4		13.7	9.8					
6		13.3	7.5					
2		12.2	11.9					
4		12.8	9.2					
6		14.8	7.5					
HARRISON (*4*), ON FRESH BASIS[2]								
	I	16.7	1.3	4.9	7.8	0.3	2.4	
	II	17.3	1.9	5.1	8.4	.2		
	III	19.8	1.5	6.9	8.3	.4	2.7	
HARRISON (*4*), DIGESTION COEFFICIENTS								
	I		64.0	62.0	61.0	45.0		
	II		65.0	58.9	54.2	66.6		
	III		60.2	71.6	64.2	60.2		
HARRISON (*4*), DIGESTIBLE NUTRIENTS								
	I		0.8	3.0	4.8	0.1		8.7
	II		1.2	3.0	4.6	.1		9.0
	III		.9	4.9	5.3	.2		11 3

[1] Includes crude fat.
[2] Harrison presented original data on fresh basis.

In separate investigations at the Florida Agricultural Experiment Station by Blaser, Kirk, and Arnold to evaluate Napier grass under rotational grazing with beef and dairy cattle, samples were plucked at regular intervals from protected quadrats in a manner to simulate grazing in the remainder of the field. These quadrats were moved at regular intervals. The harvested material was used to represent the composition of the grass as grazed. Proximate analyses were made by methods of the Association of Official Agricultural Chemists (*1*). Calcium, magnesium, and phosphorus were determined by the method of Morris, Nelson, and Palmer (*5*).

RESULTS OF EXPERIMENTS

The composition of the Napier grass as used in the digestion trials, and from the separate grazing tests conducted by Blaser, Kirk, and Arnold for a 2-year period, are presented in table 2. The ranges among samples used to compute the average composition were:

TABLE 2.—*Composition, coefficients of digestibility of nutrients, and digestible nutrient content of Napier grass leaves harvested in a manner to simulate grazing*

Source of Samples	Dry matter	Crude protein	Crude fiber	Nitrogen free extract	Crude fat	Ash	Total digestible nutrients
	COMPOSITION OF FRESH GRASS						
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Digestion trials [1]	20.8	2.7	6.0	9.4	0.9	1.8	----------
Grazing trials [2]	21.4	2.8	6.5	9.8	0.7	1.6	----------
	COEFFICIENTS OF DIGESTIBILITY						
Steer:							
E 58	64.6	65.0	66.3	68.8	56.0	----------	----------
E 69	66.6	65.1	70.1	68.8	59.2	----------	----------
E 71	66.0	65.2	65.2	70.1	57.2	----------	----------
E 76	66.5	64.2	69.5	70.4	58.1	----------	----------
Average	66.0	65.0	68.0	70.0	58.0	----------	----------
	DIGESTIBLE NUTRIENTS						
Digestion trials [1]	----------	1.8	4.1	6.6	0.5	----------	13.7
Grazing trials [2]	----------	1.8	4.4	6.9	.4	----------	14.0

[1] Digestion trial records are based on analyses of 21 samples taken Aug. 11 to 30, 1938.
[2] 23 samples of Napier grass represent 2 entire seasons for 1 area, and 1 season for a second area under rotational grazing; unpublished data used by permission of Blaser, Kirk, and Arnold.

Dry matter 16.5 to 24.0 percent, crude protein 9.9 to 17.5 percent, crude fiber 26.5 to 33.6 percent, crude fat 2.4 to 4.5 percent, and ash 6.0 to 8.4 percent. The ash content is less than half that of the values given in table 1. The average content of calcium, of magnesium, and of phosphorus were respectively, 0.50, 0.18, and 0.35 percent. The dry matter and protein content of the leaves is higher than reported by Wilsie, Akamine, and Takahashi (*10*), Paterson (*8*, *9*), or Harrison (*4*), indicating that the materials are not altogether comparable.

Each of the four steers consumed approximately 15.5 pounds of dry matter daily, or about 75 pounds of fresh grass, which was sufficient to maintain their weight or to cause slight gains. The daily

feed intake and fecal output of the steers were quite uniform throughout the trial. The average digestion coefficients for the respective steers are presented in table 2.

The values for digestible nutrient content presented in table 2 were found by applying the digestion coefficients to the analyses in the same table. The fresh grass, having a dry matter content of 21.4 percent, averaged 1.8 percent digestible crude protein and 14.0 percent total digestible nutrients, or 8.5 and 65.7 percent respectively on a moisture-free basis.

The digestibility of fresh Napier grass and other comparable forages is presented in table 3. Napier grass leaves as grazed were more digestible than the whole plant as determined by Harrison but were not as digestible as mixed pastures grasses and clovers from closely grazed fertile pasture. However, they compared favorably with mixed immature grasses; were more digestible than bluegrass, redtop or timothy, and much more digestible than the more mature Napier grass plant after ensiling.

TABLE 3.—*Digestion coefficients of the nutrients in Napier grass as compared with those in other pasture grasses reported by Morrison (6, Appendix, table 1)* [1]

Forage	Number of trials	Crude protein	Crude Fiber	Nitrogen-free extract	Crude fat
Napier grass leaves [2]	4	65	68	70	58
Napier grass [2]	3	63	64	60	57
Native bluegrasses	2	64	45	60	50
Grasses, mixed, immature	2	70	66	75	62
Orchard grass	3	60	60	55	54
Pasture grasses and clovers, mixed, from closely-grazed, fertile pasture	51	77	76	78	56
Redtop	3	61	61	62	50
Sudan grass	4	72	76	69	72
Timothy	3	48	56	66	53

[1] Data used by permission of the author.
[2] From table 2 of this paper.
[3] Harrison (4).

SUMMARY

Napier grass leaves as grazed rotationally by cattle provide pasture which is comparable in nutrient content with other pasture grasses. The digestion coefficients were found to be: For crude protein 65, for crude fiber 68, for nitrogen-free extract 70, and for crude fat 58, as compared with average coefficients of 63, 64, 60, and 57, respectively, as determined by Harrison. The fresh grass provided 1.8 percent of digestible crude protein and 14 percent of total digestible nutrients, or 8.5 and 65.7 percent respectively, on a moisture-free basis.

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